Alerts, Notices, and Case Reports

Identifying Multiple Isolates Through the Comparison of Genomic DNAs in a Patient With Infected Hip Prosthesis

FRANÇOISE PERDREAU-REMINGTON, MD, PhD
San Francisco, California
DOUGLAS R. RANK, MD
FRED A. LOPEZ, MD
STUART B. GOODMANN, MD
Stanford, California

TOTAL HIP REPLACEMENTS are performed in approximately 120,000 individuals in the United States each year.[1] Approximately 10% of these prostheses will need to be modified after 10 years due to loosening.[2] Pain from aseptic loosening is often difficult to differentiate from pain associated with prosthetic hip infection, a devastating complication ranging in incidence from 0.06% to 0.69%.[3] The most common isolates from presumptively infected hip prostheses are coagulase-negative staphylococci.[1,4,5] Unfortunately, it is difficult to interpret a positive culture with single or even multiple coagulase-negative staphylococci isolates from a prosthetic hip aspirate or surgical specimen because the presence of coagulase-negative staphylococci, a normal human skin commensal, may represent contamination and not necessarily infection.

The clinical presentation of prosthetic joint infection is highly variable, ranging from acute septic arthritis with toxic systemic signs and symptoms to indolent loosening of the prosthesis with chronic pain. Early infection is most commonly acquired by contamination during surgery; late infection usually is secondary to a hematogenous spread to the prosthesis from a distant source (such as intravascular line sepsis) or focus of an infection.[6] Late infections may also be due to coagulase-negative staphylococci introduced at the time of surgery. These coagulase-negative staphylococci survive by forming a glycocalyx, or slime, on the implant surface, and they result in clinical infection.[7–9]

(Perdreau-Remington F, Rank DR, Lopez FA, Goodmann SB. Identifying multiple isolates through the comparison of genomic DNAs in a patient with infected hip prosthesis. West J Med 1998; 168:128–130)

From the EPI-Center, Division of Infectious Diseases, San Francisco General Hospital, University of California (Dr Perdreau-Remington); the Department of Medicine and Division of Infectious Diseases, Stanford University Medical Center, Stanford, California (Drs Rank and Lopez); and the Department of Functional Restoration, Division of Orthopedic Surgery, Stanford University Medical Center, Stanford, California (Dr Goodmann).

Reprint requests to Francoise Perdreau-Remington, Director, Molecular Epidemiology Research Lab, USCF/SFGH Epidemiology and Prevention, San Francisco General Hospital, San Francisco, CA 94110.

Coagulase-negative staphylococci—more specifically, *S. epidermidis*—are the most commonly isolated bacterial species from prosthetic hip aspirates or intraoperative surgical specimens in patients with pain or loosening of the prosthesis. This is true for both hip aspiration and revision surgical procedures.[10–12]

Many criteria have been used to improve the predictive value of coagulase-negative staphylococci isolates from a prosthetic hip as a cause of loosening, infection, or both. No formula or method proposed has resulted in better than approximately 70% sensitivity or specificity,[13] and even percentages such as this are frequently difficult to substantiate with the data provided in papers published on this subject. For example, in a 1994 study designed by Steckelberg and Osmon,[13] four conditions were determined that indicate the presence of prosthetic hip infection. The sole microbiological condition was two or more positive cultures from joint aspirates or intraoperative cultures. Even this rather strict condition did not establish a given isolate as the definitive cause of infection because of its inability to establish clonality.

Since coagulase-negative staphylococci are low-virulence organisms that may represent contamination in as many as 50% of operative specimens,[14,15] more accurate methods are needed to assist in interpreting the clinical significance of these isolates. Recently we and others[16,17] have promoted the use of molecular methods to investigate the epidemiology and clinical significance of these coagulase-negative staphylococci isolates. We report the successful use of pulsed-field gel electrophoresis of restricted genomic DNA to establish the clonal identity of coagulase-negative staphylococci isolates from the blood and hip of a patient with an infected prosthesis.

Report of a Case

The patient, a 67-year old woman, underwent an uncemented left total hip arthroplasty in 1985 after a motor vehicle accident resulted in nonunion of a left femoral neck fracture with associated osteonecrosis of the left femoral head. Her postoperative course was uncomplicated, and she remained well until 1994, when she began experiencing left hip pain. A radiographic evaluation showed changes consistent with loosening of the hip prosthesis, but before revision surgery could be performed, she began running a fever and experienced an increased intensity of joint pain. Fluid obtained from aspiration of her left hip contained 202,500 leukocytes per microliter (90% granulocytes), and a Gram stain showed many polymorphonuclear leukocytes and few Gram-variable cocci. The erythrocyte sedimentation rate was elevated to 37 mm per hour. Cultures of the patient's blood and joint fluid grew *Staphylococcus epidermidis*. On removal of the infected left hip prosthesis, a Gram-

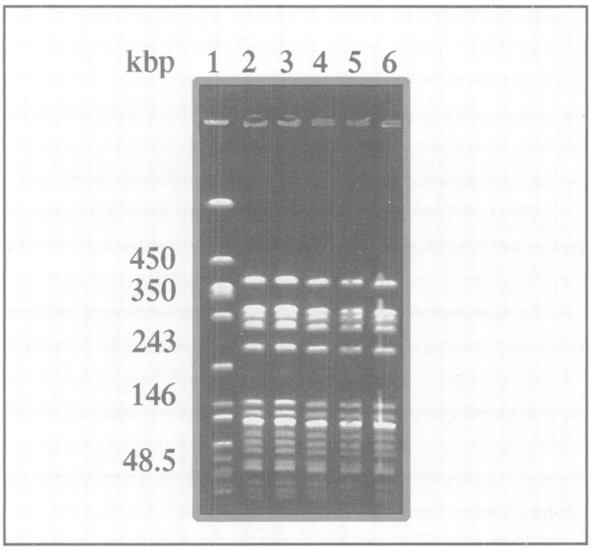

Figure 1.—Pulsed-field gel electrophoresis of chromosomal DNA of *S. epidermidis* isolates from two blood cultures, pre-operative aspirate, and intraoperative femur and acetabulum cultures, after digestion with *Sma* I (lanes 2–6). Lane 1 shows Lambda concatemers.

stained smear of purulent discharge from the patient's hip joint showed Gram-positive cocci with subsequent growth of *Staphylococcus epidermidis* in culture. A histopathologic examination of the soft tissue and bone showed inflammation consistent with an abscess and no evidence of acute or chronic osteomyelitis.

Antibiotic therapy was begun and initially consisted of intravenous cefazolin and gentamicin. Later, it was modified to cefazolin alone, and the patient was afebrile by postoperative day 5. No evidence of endocarditis was discernible through transesophageal echocardiography or a physical examination. After six weeks of intravenous antibiotic therapy, follow-up evaluation of a fluid aspirate from her left hip showed the presence of only 472 leukocytes per microliter and few polymorphonuclear leukocytes; a Gram stain showed no bacteria, and there was no growth in culture. Three months after the removal of the prosthetic hip, a resection arthroplasty was performed, at which time no intraoperative or microbiologic evidence of infection was seen.

The coagulase-negative staphylococci isolates from the blood cultures, the pre-operative hip aspirate, and the three intraoperative specimens were identified as *Staphylococcus epidermidis*. All six isolates were susceptible to penicillin, oxacillin (methicillin-susceptible), clindamycin, erythromycin, trimethoprim/sulfamethoxazole, tetracycline, ciprofloxacin, and vancomycin. An analysis of the genomic DNA of 5 of the 6 isolates (one isolate was lost) using pulsed-field gel electrophoresis revealed identical fingerprints. Restriction digestion with the enzyme *Sma* I generated DNA products in the range of 50 kb to 550 kb; the enzyme *Ksp* I generated products in the range of 10 kb to 250 kb after restriction digestion. The chromosomal restriction pattern obtained after *Sma* I digestion is shown in Figure 1.

Discussion

In a patient with pain at the site of a hip prosthesis but without clinical or laboratory signs suggestive of infection, the isolation of coagulase-negative staphylococci from one or more hip aspirate is frequently used to justify surgical intervention for presumed infection. In our experience, a consultation with the infectious diseases department is often requested to assist in evaluating the significance of such isolates because obvious purulence is not usually present. The decision to start treatment becomes critical because of the intensive and relatively long-term intravenous antibiotic treatment that is begun and often believed to be the safest course of action in these cases.

Treatment involves prolonged hospitalization, high costs, increased risk of toxicity, and inconvenience to the patient. In the absence of gross and histologic evidence of acute infection, it is problematic to conclude that multiple isolates from hip aspiration or from surgical sites are the cause of prosthetic hip loosening and infection. Identifying the multiple isolates from the same clonal lineage, however, would help justify such a conclusion. If isolates of coagulase-negative staphylococci from different sites and at different times can be shown to be identical through genetic fingerprinting (as in the case presented here), then strong evidence exists for their etiological role in prosthetic hip loosening.

Several attempts have been made to use typing methods to evaluate the role of coagulase-negative staphylococci isolated from loose prosthetic hips at the time of revision surgery. These methods include antibiotic susceptibility patterns, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of extracellular products, plasmid analysis, and ribotyping. Each of these methods is subject to problems with interpretation and accuracy. Therefore, as one study illustrates,[10] antibiotic sensitivity profiles should be interpreted with caution when used to determine clonal identity. When used for molecular typing, SDS-PAGE is cumbersome, and protein profile reproducibility may be altered by the proteolytic activity of the strain.

A recent report focused on plasmid analysis, which has long been documented to not always be reliable as a marker for strain identification because isolates can rapidly exchange plasmids or acquire new ones.[18] In another case,[11] a single ribotype of *S. epidermidis* (type I) accounted for approximately half of all *S. epidermidis* cultures examined in a 29-month period; in one instance, ribotyping helped to identify a predominant and persisting ribotype of *S. epidermidis*. Ribotyping unfortunately does not identify strains at the clonal level because it detects highly conserved regions of ribosomal RNA. Another study[19] shows that there are similarities between ribotypes of different species; therefore, intraspecific fragment pattern diversity can interfere

with interspecific discrimination. This is an obvious limitation of this method.

We were unable to identify a distant source for the infection in our patient. We cannot rule out the possibility that her hip infection resulted from hematogenous spread of the coagulase-negative staphylococci; however, it appears more likely that her infection originated directly from her hip.

In contrast to the methods mentioned above, pulsed field gel electrophoresis of genomic DNA is considered the reference technique for strain characterization at the genomic level. Applicable to all organisms that can be grown in culture, pulsed-field gel electrophoresis is a useful tool in determining categories of genetic and epidemiological relatedness; this method is used extensively to investigate outbreaks of infection as well as hospital environmental contamination.[20] Isolates are considered to be identical if they have exactly the same electrophoretic pattern and to be clonally related if they show a difference of three or fewer bands between strains.[21] In the case presented here, we have used pulsed-field gel electrophoresis to document the clonal identity of five strains of coagulase-negative staphylococci recovered from our patient at different times and from different sites. We employed genetic fingerprinting using restriction fragment length polymorphism (RFLP) analysis to determine whether *S. epidermidis* isolates were clonal in origin. Confirmation of a single clone was illustrated by identical RFLPs following pulsed field gel electrophoresis of restriction digests with two enzymes, *Sma* I and *Ksp* I. Thus, RFLP analysis appears to be useful in determining the significance of coagulase-negative staphylococci from patients with prosthetic hip loosening associated with suspect infection.

The use of a molecular epidemiologic tool, pulsed-field gel electrophoresis, to type the *S. epidermidis* isolates from blood, pre- and intraoperative aspirates, and bone from the femur and acetabulum of our patient confirmed that all *S. epidermidis* isolates were identical. The etiology of the infection would appear to be more certain in patients demonstrating the same clone in multiple specimens, but the dilemma of finding multiple clones remains puzzling. The results of our investigation support the clinical and laboratory observations that these isolates were the etiology of the patient's hip infection and the likely cause of the loosening of her hip.

Acknowledgment

The authors wish to thank Nhung Huynh for her excellent technical assistance.

REFERENCES

1. Harris WH, Sledge CB. Total hip and total knee replacement. N Engl J Med 1990; 323:725–730

2. Mulroy WF, Estok DM, Harris WH. Total hip arthroplasty with use of so-called second-generation cementing techniques: a fifteen-year-average follow-up study. J Bone Joint Surg Am 1995; 77:1845–1852

3. Fitzgerald RH. Diagnosis and management of the infected hip prosthesis. Orthopedics 1995; 18:833–835.

4. Fitzgerald RH. Total hip arthroplasty sepsis: prevention and diagnosis. Orthop Clin N Am 1992; 23:259–264

5. Hope PG, Kristianson KG, Norman P, Elson RA. Deep infection of cemented total hip arthroplasties caused by coagulase-negative staphylococci. J Bone Joint Surg Br 1989; 71:8551–8555

6. Dougherty SH. Microbiology of infection in prosthetic devices. *In* Wadstrom T, Eliason I, Holder I, Ljungh A (Eds): Pathogenesis of Wound and Biomaterial-Associated Infections. London, Berlin, Heidelberg, New York: Springer-Verlag, 1990, pp 375–390

7. Gristina AG, Costerton JW. Bacterial adherence to biomaterials and tissue: the significance of its role in clinical sepsis. J Bone Joint Surg Amer 1985; 67:264–273

8. Dobbins JJ, Seligson D, Raff MJ. Bacterial colonisation of orthopedic fixation devices in the absence of clinical infection. J Infect Dis 1988; 158:203–205

9. Peters G, Schumacher-Perdreau F, Jansen B, Bey M, Pulverer G. Biology of *S.epidermidis* extracellular slime. *In* Pulverer G, Quie PG, Peters G (Eds): Pathogenicity and Clinical Significance of Coagulase-Negative Staphylococci. Zentralbl Baktriol Suppl 1987; 16:15–32

10. Kristinsson KG, Hope P, Norman P, Elson RA. Deep infections associated with total hip arthroplasties caused by coagulase-negative staphylococci: pathogenesis and microbial diagnosis. *In* Wadstrom T, Eliason I, Holder I, Ljungh A (Eds): Pathogenesis of wound and biomaterial-associated infections. London, Berlin, Heidelberg, New York, Paris, Tokyo,Hong Kong: Springer-Verlag, 1990, pp 329–337

11. Crichton PB, Anderson LA, Phillips G, Davey PG, Rowley DI. Subspecies discrimination of staphylococci from revision arthroplasties by ribotyping. J Hosp Infect 1995; 30:139–147

12. Perdreau-Remington F, Stefanik D, Peters G, Ludwig C, Ruett J, Wenzel R, Pulverer G. Microbial ecology of explanted prosthetic hips: a four year prospective study of 52 patients with aseptic prosthetic joint loosening. Eur J Clin Microbiol Inf Dis 1996; 15:60–65

13. Steckelberg JM, Osmon DR. Prosthetic joint infections. *In* Bisno AL, Waldvogel FA (Eds): Infections associated with indwelling medical devices (2nd ed) Washington DC: American Society for Microbiology, 1994, pp 259–283

14. Lidgren L. Low virulent bacteria in joint implant infection. *In* Wadstrom T, Eliason I, Holder I, Ljungh A (Eds): Pathogenesis of wound and biomaterial-associated infections. London, Berlin, Heidelberg, New York, Paris, Tokyo,Hong Kong: Springer-Verlag, 1990, pp 363–367

15. Tigges S, Stiles RG, Meli RJ, Roberson JR. Hip aspiration: a cost-effective and accurate method of evaluating the potentially infected hip prosthesis. Radiology 1993; 189:485–488

16. Schumacher-Perdreau F, Jansen B, Peters G, Pulverer G. Typing of coagulase-negative staphylococci isolates from foreign body infections. Eur J Clin Microbiol Infect Dis 1988; 7:270–273

17. Lyytikäinen O; Saxén H; Ryhänen R; Vaara M; Vuopio-Varkila J. Persistence of a multiresistant clone of *Staphylococcus epidermidis* in a neonatal intensive-care unit for a four-year period. Clin Infect Dis 1995; 20:24–9

18. Naidoo J, Noble WC. Skin as a source of transferable antibiotic resistance in coagulase-negative staphylococci. Zetrlbl Bakteriol (A Suppl) 187; 16:225–232

19. Izard NC, Hachler H, Grehn M, Kayser FH. Ribotyping of coagulase-negative staphylococci with special emphasis on intraspecific typing of *Staphylococcus epidermidis*. J Clin Microbiol 1992; 30:817–823

20. Perdreau-Remington F, Stefanik D, Ruckdeschel W, Pulverer G. *Staphylococcus haemolyticus* on the hands of health care workers: a route of transmission or a source? J Hosp Infect 1995; 31:195–203.

21. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE , Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial typing. J Clin Microbiol 1995; 33:2233–2239

The Detection of Recurrent Sarcoidosis by FDG-PET in a Lung Transplant Recipient

CHARN KIATBOONSRI, MD
STEVE C. RESNICK, MD
KEVIN M. CHAN, MD
RICHARD G. BARBERS, MD
CHARLES C. MARBOE, MD
ALI KHONSARY, MD
SILVERIO M. SANTIAGO, MD
OM P. SHARMA, MD
Los Angeles, California

END-STAGE LUNG DISEASE secondary to sarcoidosis is an accepted indication of the need for lung transplantation. Despite the apparent successful outcomes of lung transplant patients, several case reports have emerged regard-